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THE EFFECTS OF *PHYLLANTHUS AMARUS* EXTRACT ON THE REPRODUCTIVE FUNCTIONS OF MALE RATS

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By

RONNIE KUEH BOON LIN

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master o Science in the Faculty of Science and Environmental Studies Universiti Putra Malaysia

February 2001

Dedicated to my mother,
who has given me so much love
and encouragement throughout my life.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science.

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Chairman: **Dr. Hamdan Hj. Mohd. Noor**

Faculty: **Science and Environmental Studies**

Global population has grown at such an alarming rate that it is critically straining the planet's resources. Thus regulating human fertility with an effective and inexpensive remedy, for example from plant sources is an attractive proposition. In this study, *Phyllanthus amarus* aqueous extract was given orally at 50, 100, 250 and 500mg/kg body weight daily for 90 days to male rats to investigate its effect on the male reproductive function. Clomiphene citrate (a known male anti-fertility agent) was given orally at 5mg/100g b.w. for comparison. The extract treatment lowered the fertility index (100, 250 and 500 mg/kg b.w.) but litter size was not affected. Testicular (500mg/kg b.w. group) and prostate (all extract treated groups) weights were significantly reduced. Epididymal sperm count and viability were significantly lowered (500mg/kg b.w. group) without affecting motility and morphology. Testicular cholesterol showed a trend increase but was not significantly different compared to control whereas the mean volumetric proportion of testicular spermatozoa (500mg/kg b.w. group) was significantly lower. Seminiferous tubules of rats given the highest dose showed interference in spermatogenesis at the spermatid

level with the presence of degenerated spermatids in the lumen. In clomiphene treated rats, fertility was suppressed and the testis, epididymis, seminal vesicles and prostate were severely affected. Epididymal sperm count dropped to less than 2% of control while motility and viability were also as severely affected. Meanwhile sperm morphology revealed abnormalities of the head, neck and tail. Testicular histology showed progressive regression of the seminiferous tubules with most tubules devoid of spermatozoa and spermatid while testicular cholesterol was much higher compared to control. These results suggest that *Phyllanthus amarus* had a mild inhibitory effect on the male reproductive function as compared to clomiphene. These effects were reversible upon cessation of treatment. The extract possibly exerted an estrogen-like effect on the male reproductive system.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

KESAN EKSTRAK *PHYLLANTHUS AMARUS* KE ATAS FUNGSI PEMBIAKAN TIKUS JANTAN

Oleh

RONNIE KUEH BOON LIN

Febuari 2001

Pengerusi: **Dr. Hamdan Hi. Mohd. Noor**

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Penduduk dunia kini telah bertambah dengan begitu pesatnya sehingga memberi bebanan yang kuat terhadap sumber semulajadi bumi. Oleh itu, adalah penting untuk tahap kesuburan manusia dikawal dengan suatu cara yang murah dan berkesan yang mungkin boleh diperolehi dari sumber tumbuhan. Dalam kajian ini, ekstrak akueus *Phyllanthus amarus* telah diberi kepada tikus secara minuman mengikut dos 50, 100, 250 dan 500 mg/kg berat badan (b.b.) selama 90 hari untuk mengkaji kesannya ke atas sistem pembiakan jantan. Clomiphene sitrat (sejenis agen anti kesuburan jantan) diberi mengikut dos 5 mg/100g berat badan sebagai perbandingan. Rawatan ekstrak telah berjaya mengurangkan indeks kesuburan (100, 250 dan 500 mg/kg b.b.) tetapi bilangan anak yang dilahirkan tidak berbeza secara bererti. Berat testis (500 mg/kg b.b.) dan kelenjar prostat (semua kumpulan rawatan ekstrak) telah menurun secara bererti. Bilangan sperma epididimis dan kemandirian sperma (500 mg/kg b.b.) juga telah menurun secara bererti tetapi kecerdasan pergerakan dan morfologi sperma tidak terjejas. Kepekatan kolesterol testis menunjukkan corak peningkatan tetapi tidak berbeza secara bererti berbanding kawalan manakala isipadu purata bahagian

spermatozoa (500 mg/kg b.b.) menurun secara bererti. Tubul seminiferus tikus yang diberi dos ekstrak tertinggi menunjukkan kesan gangguan pada spermatogenesis pada tahap spermatid dengan kehadiran spermatid cacat di dalam ruang lumen. Dalam tikus yang dirawat dengan clomiphene, kesuburan tikus jantan terjejas manakala testis, epididimis, vesikel seminal dan kelenjar prostat menerima kesan yang teruk daripada rawatan tersebut. Bilangan sperma epididimis telah menurun sehingga kurang dari 2% paras kawalan manakala kecerdasan pergerakan dan kemandirian sperma turut terjejas dengan teruk. Dari aspek kajian morfologi sperma terdapat kehadiran sperma yang cacat pada bahagian kepala, leher dan ekor. Kajian histologi menunjukkan tanda-tanda regresi tubul seminiferus dengan ketidakhadiran spermatozoa dan spermatid pada kebanyakan tubul. Kandungan kolesterol testis juga didapati sangat tinggi berbanding dengan kawalan. Daripada keputusan kajian ini, ekstrak akueus *P. amarus* didapati dapat memberi kesan negatif kepada sistem pembiakan jantan tetapi kesan ini kurang kuat berbanding clomiphene. Walaubagaimana pun kesan ini hilang selepas rawatan ditamatkan. Ekstrak ini berkemungkinan mempunyai kesan seakan-akan hormon estrogen ke atas sistem pembiakan jantan.

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I certify that an Examination Committee met on 6th February 2001 to conduct the final examination of Ronnie Kueh Boon Lin on his Master of Science thesis entitled 'The Effects of *Phyllanthus amarus* Extract on the Reproductive Functions of Male Rats' in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and was accepted as fulfilment of the requirement for the degree of Master of Science.

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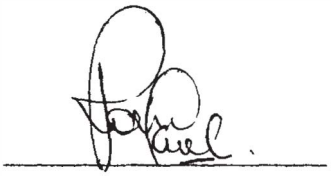
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



RONNIE KUEH BOON LIN

Date:

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LIST OF ABBREVIATIONS

b.w.	body weight
°C	degrees Celcius
g	grams
g	gravitational force
M	molar
mM	millimolar
mg	milligram
ml	milliliters
ng	nanogram
nm	nanometer
OD	optical density
p	probability of an event due to chance alone
pH	$-\log_{10}[H^+]$
rpm	rotation per minute
s.e.m	standard error of mean
µl	microliters

CHAPTER I

INTRODUCTION

Population explosion

Recorded history offers no parallels to humanity's successes at survival and reproduction. In retrospect, it is phenomenal that the world's population mushroomed from 1 billion people in the year 1800 to more than 5 billion by the year 1900. It is even more staggering to imagine planet earth with yet another 3 billion within the next 30 years – as projected by the United Nations (Sadik, 1991).

Many people in different professions have begun to recognize that there may be too many human beings in the world. Population has become a controversial subject, with some people predicting doom and others arguing that there is nothing to worry about. Although the experts themselves often disagree, one aspect of the population explosion is non-controversial: the world cannot support an infinite number of persons (Hartley, 1972). The accelerating growth in world population running parallel with world economic growth has brought stresses and strains upon the global environment. Almost two centuries ago, the English economist, Thomas Malthus, in his *Essay on the Principle of Population* drew attention to the growing population and suggested that the world's peoples were increasing more rapidly than its food supply. Many of the problems of our time - malnutrition, disease, illiteracy, unemployment, urbanization, the energy crisis, environmental degradation, political problems, to name but a few – are direct outcome of population growth (Robinson,

1981). To this end, much attention has been paid by many international bodies to one of the key measures – birth control or contraception.

History of fertility regulation

Throughout history there has been a desire to control conception, at least to the level accepted by a particular society in which the woman lived (Llewellyn-Jones, 1974). Many birth control practices are at least as old as recorded history. The Old Testament contains obvious references to the practice of withdrawal, or *coitus interruptus* (removal of the penis from the woman's vagina before ejaculation) (Ehrlich & Ehrlich, 1972).

The first method of contraception by the Egyptians is in the Petrie papyrus of 1850 BC in which a variety of magical potions are mentioned, but which also suggests the use of a vaginal plug formed from crocodile dung and honey, which was to be inserted into the vagina prior to coitus. The Ebers papyrus, written 300 years later also mentioned a vaginal pessary of lint soaked in the juice from the tips of acacia shrubs, mixed with honey. The honey would form a film penetrated with difficulty by the spermatozoa and the acacia tips contain gum arabic that releases lactic acid, a fairly efficient spermicide. Mechanical methods of contraception were unknown in Europe, although in 1564 Fallopius, an Italian anatomist, had recommended a linen covering of the glans penis as a protection against syphilis, which was then endemic throughout Europe. At that time, the condom (as it is now called) was made from the caecum of sheep and was drawn onto the erect penis to cover both glans and shaft (Llewellyn-Jones, 1974).

Although for centuries contraception was left up to males, who used *coitus interruptus* and the condom, attention to chemical methods for men began in earnest with clinical trials only in 1971. A difficulty encountered in the field of contraceptive research in the male is the small number of links in the reproductive chain of events, compared to the number of vulnerable points identified in the female system. The focus has been suppressing the production of sperm through the action of synthetic male hormones in a manner analogous to the suppression of egg production and ovulation in the female with the pill (Hauser, 1979).

Fertility regulation today

Several methods to control human fertility are available today. However, an ideal contraceptive that is safe, inexpensive, totally effective, easily reversible and without side effects is yet to be developed. Oral contraceptives, intrauterine devices, male and female sterilizations, barrier contraceptives, medical termination of pregnancy, rhythm and symptothermal methods and *coitus interruptus* are presently being used to control births (Salunkhe *et al.*, 1989). Despite historical interest in methods for male contraception, the only current means for the public are interrupted coitus, vasectomy and condom use. The condom is generally described as a 'male method' and apart from *coitus interruptus*, it is the only reversible method available to them (Paulsen *et al.*, 1994).

Coitus interruptus or male withdrawal is certainly the oldest method of contraception. It is the simplest method to comprehend, requires no professional advice or appliances, costs nothing and cannot be forgotten. The side effects ascribed

to *coitus interruptus*, as with many other contraception method go back to moral condemnation of the method and sometimes what is really being condemned is contraception itself (Potts, 1994). Vasectomy, or more precisely vas sectioning and occlusion, has become a popular elective procedure for permanent male contraception. Whereas failure rates of temporary methods of contraception are additive year by year, the 0.1% chance of recanalization decreases after the first year of vasectomy approaching zero, making it the most effective method for permanent contraception (Davis & Pollack, 1994).

One strategy for controlling male fertility is to suppress production of the hormones that support spermatogenesis. Perhaps the biggest obstacle to this approach is that testosterone supports several physiologic functions, including not only maintenance of sperm production, but also of libido and potency, external male characteristics and physical strength. Progestogens, estrogens and androgens, LHRH analogue treatments and suppression of gonadotropins such as FSH all have been attempted as approaches to regulation of male fertility. Essentially, every compound that blocks sperm production by suppressing testosterone synthesis potentially produces unacceptable symptoms of hypogonadism and many investigators believe that practical application of such compounds would require suitable androgen replacement therapy (Bartke *et al.*, 1985).

Birth control or anti-fertility vaccines, the idea that circulating antibodies or cellular immune effector cells might be induced by a vaccine at sufficient levels and with sufficient specificity to interrupt reproductive processes and sustain a period of infertility, without side effects, has gained considerable scientific strength in recent

years (Herr *et al.*, 1994). Talwar (1997) clinically tested the first contraceptive vaccine for women, the human chrionic gonadotropin (hCG) vaccine and found it to be safe. The first ever contraceptive vaccine for men was by Moudgal *et al.* (1997) which was the heterologous follicular stimulating hormone (FSH). Other vaccine developments are against sperm and egg antigens (Naz *et al.*, 1995). However, one common thread that ran through all these studies was that none of these vaccines were effective in all the individuals (Anand Kumar, 1997).

Plants in fertility control

The development of new methods of fertility regulation from plants is an attractive proposition, particularly for developing countries because traditional medicine is still widely practiced there and many of the plants with anti-fertility activity are found there (World Health Organisation, 1998). The role of plants and their products in the induction of reversible male infertility in experimental animals as well as in humans has drawn the attention of modern researchers (Lohiya *et al.*, 1994). Plants contain a variety of chemical constituents. Some of these chemicals are specific for a species or a group of species, while others are common in all plants. The medicinal value of many plants has been attributed to the presence of certain chemicals. Some of the medicinally active plant constituents have been used in the form of crude extract preparations by ancient civilizations. Primitive societies or tribes have used many plant products for controlling births (Salunkhe *et al.*, 1989).

Similarly the World Health Organization (WHO) has set up a task force on plants for fertility regulation, the strategic plan of which is to identify novel drug prototypes

found in plants which have been alleged to have fertility regulating properties. Compounds that are being sought in particular are those orally active, non steroidal, non estrogenic, safe and effective for the prevention or disruption of implantation in women and those that will inhibit spermatogenesis or interfere with sperm maturation in men (Griffin, 1988, cited by Xiao & Wang, 1991). Plants do indeed contain a variety of constituents that can be demonstrated to have fertility regulating properties. For the male who wishes to take responsibility for contraception, gossypol (derived from the oil of cottonseed) is still the most promising agent from natural resources (Coutinho *et al.*, 2000).

Many of the drugs in the market today contain one or more of the active principles derived from higher plants. However, the plants or plant products having anti-fertility activity have not been investigated extensively to the point of developing an acceptable anti-fertility drug. Although some plant products have been found to be useful in controlling fertility in women, practically none of the plant products tested so far has found a general acceptance for controlling male fertility (Salunkhe *et al.*, 1989).

Objective of study

The objective of this study is to

(i) determine the effects of *Phyllanthus amarus* aqueous extract on the male rat reproductive system, and if the effect is apparent:

(ii) determine the possible reversal of this effect, and

(iii) determine the possible mechanism of action.

CHAPTER II

LITERATURE REVIEW

Regulation of male fertility

The male reproductive tract

The testes contain two functional units: a network of tubules for the production and transport of sperm to the excretory-ejaculatory ducts and a system of interstitial or Leydig cells that contain the enzymes for the production of androgens. Spermatogenic tubules are composed of germ and Sertoli cells. Tight junctions between the Sertoli cells at a site between the spermatogonia and the primary spermatocyte form a diffusion barrier that divides the testis into two functional compartments, the basal and the adluminal (Griffin, 1993). The barrier between these two compartments has limited permeability to macromolecules, analogous to the blood-brain barrier and other epithelial barriers (Neaves, 1977).

The basal compartment consists of the Leydig cells, the boundary tissue of the tubule, and the outer layers of the tubules containing the spermatogonia. The adluminal compartment contains the inner two thirds of the tubules, including primary spermatocyte and more advanced stages of spermatogenesis. The cytoplasm of the Sertoli cells encompasses the differentiating spermatocytes and spermatids so that spermatogenesis takes place within a network of Sertoli cell cytoplasm (Griffin, 1993). The Leydig cells contain lipid droplets with esterified cholesterol that serves as a reservoir of substrate for testosterone synthesis (Freeman, 1987).